MAIZE GENETICS

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CONTINUATION OF THE STUDY OF THE INDUCTION OF NEW MUTANTS IN CHROMOSOME 9

The study of the induction of new mutants in the short arm of chromosome 9, following the chromatid and chromosome breakage cycles involving this arm, is nearing completion. At present, nine new mutant types have been isolated that are located in the short arm of chromosome 9. The mutants pale-yellow seedling (*pyd*), white seedling (*wd*), and yellow-green seedling and plant (*yg*) have been repeatedly induced by the breakage-fusion cycles. Only one each of the following mutants has been obtained: bronze aleurone, virescent seedling, pale-green seedling, small-kernel, narrow-leaf, and light-green plant. Only in one branch of this study was an attempt made to determine the proportion of all newly arising seedling or kernel mutants that are located in chromosome 9. Three hundred and eighty-three plants that showed the chromosome type of breakage cycle in their early developmental stages were self-pollinated and the kernels and seedling progeny observed. Thirty-two newly arising stable (nonvariegating, see below) recessive mutants were observed. Twenty-four of these were located in chromosome 9 (5 *pyd*, 11 *wd*, 5 *yg*, 1 small-kernel, 1 narrow-leaf, 1 light-green); four of the new mutants were not located in chromosome 9, although one of them showed an unexpected relation to a previously known mutant in chromosome 9; tests of the four remaining new mutants have not been completed. In the majority of the parent plants, only one of the two chromosomes 9 could be thoroughly tested for the presence of a new mutant, because of the frequent lack of transmission through the pollen of one of the altered chromosomes 9.

Cytological observations of the chromosome 9 carrying a newly arising mutant have been made in the case of 13 *pyd* mutants, 11 *wd* mutants, 8 *yg* mutants, and the small-kernel, pale-green, and bronze mutants. In 12 of the *pyd* mutants, all of the *wd* mutants, the bronze mutant, and the small-kernel mutant, a chromosomal deficiency or an obvious alteration was evident at the "locus" of the mutant.

In the remaining cases examined, the particular chromosomal event that gave rise to the mutation could not be determined with certainty.

One of the four new mutants not located in chromosome 9 showed an interesting relation to the mutant *c*, which is located in the short arm of chromosome 9. The recessive mutant *c*, when homozygous, produces kernels that have no color in their aleurone layer. The dominant allele, *C*, gives rise to colored aleurone. The new mutant, a simple recessive in breeding behavior, produces small kernels with defective embryos. Plants that were *Cc* and likewise heterozygous for the small-kernel mutant were self-pollinated. Colored and genotypically colorless (*cc*) kernels and normal and small kernels segregated independently. The small kernels in the homozygous *cc* class, however, were not completely colorless, as expected. Instead, they showed a faint but distinct blush of color in the aleurone layer, closely resembling that produced by the *bz* mutant. Cytological examination has not been made to determine whether the origin of the small-kernel mutant may have involved chromosome 9 in some unusual manner. The peculiar relations of the spotted-leaf mutant, as described below, suggest this possibility.

Modification of Mutant Expression Following Chromosomal Translocation

The recessive mutant "spotted-leaf" (*spl*), in early investigations, gave evidence indicating that it was a recessive located in the short arm of chromosome 9. This was because the spotted-leaf character appeared in the appropriate chromosomal class in F_1 plants following the cross of a spotted-leaf plant to a female plant heterozygous for a relatively long but transmissible terminal deficiency of the short arm of chromosome 9. Further study of this particular mutant revealed that its inheritance is not typical of a simple recessive and that its locus cannot be placed in the short arm of chromosome 9 in the usual manner.

The spotted-leaf mutant is not detected in the young seedling stage. Chlorophyll development in the seedling usually appears quite normal. As the plant continues to grow, the chlorophyll throughout the plant changes to a bright yellowish green. Later, further changes in the chlorophyll occur. In evenly distributed, small spots throughout the leaves, the chlorophyll appears to break down still further. Thus, on a light-green leaf there appear many small yellowish spots. The mutant has consequently been called "spotted leaf." Following the production of the spots, the plant may exhibit various degrees of necrosis of the cells of the leaf, beginning at the tips of the leaves. This degeneration is accelerated by excessive heat and light. Relatively few spotted-leaf plants have survived to maturity.

The spotted-leaf mutant first appeared in the progeny of a self-pollinated plant that was being investigated for purposes other than the detection of new mutants. This parent plant had one chromosome 9 with the minute terminal deficiency that, when homozygous, produces pale-yellow (*pyd*) seedlings. This chromosome 9, of known constitution, had been introduced into the parent plant by a controlled cross. Its homologous chromosome 9 had previously been altered in constitution following a series of breakage cycles. During these breakage cycles, an additional chromosomal alteration occurred. It involved the translocation to the end of the short arm of chromosome 8 of a segment composed of six chromomeres derived from the end of the short arm of chromosome 9.

As this segment of chromosome 9, before its translocation to the short arm of chromosome 8, had been subjected to the breakage-fusion cycles, it was not an unmodified terminal segment. Its exact composition is still unknown. Cytological observations indicate that chromosome 8 could not have lost more than a chromomere from the tip of its short arm during the translocation process; if as much chromatin as this has been removed, the resulting deficiency does not interfere with pollen transmission to a marked extent, for this chromosome is readily transmitted through the pollen. (Translocations involving the broken end of chromosome 9 during its chromatid breakage cycles with other chromosomes of the complement are not infrequent. See Year Book No. 41.) The parent plant had, then, a *pyd* chromosome 9, the newly modified chromosome 9, a normal chromosome 8, and the chromosome 8 with the translocated segment. This plant was normal in appearance. The new spotted-leaf mutant as well as the expected *pyd* mutant appeared in the progeny of this plant following self-pollination.

All evidence to date indicates that the spotted-leaf character will appear whenever a plant is homozygous for any one of the *pyd*-producing deficiencies of chromosome 9 and also has one normal chromosome 8 and one chromosome 8 with the translocated segment. In other words, the chromosome 8 with the translocated segment is responsible for the appearance of the mutant spotted-leaf, but only when a short terminal homozygous deficiency is also present in chromosome 9. A simple interpretation, subject to verification, of this relatively complex genotypic expression is possible. The terminal deficiency that produces the *pyd* mutant is large enough to be cytologically detected. Possibly the segment of the tip of chromosome 9 that has been translocated to chromosome 8 is itself incomplete and has a very minute deficiency for a segment of chromatin within the limits of the larger *pyd*-producing deficiency. Thus, plants having two deficient *pyd* chromosomes 9 and the duplication carried by chromosome 8 would be homozygous deficient for a minute segment of chromosome in the tip region of chromosome 9 even smaller than that which produces the *pyd* character. If this is so, the spotted-leaf character would be expected to appear as a simple recessive mutant, located close to the tip of the short arm of chromosome 9, in the progeny tests of some plants whose chromosomes 9 have undergone the breakage cycles. It would be expected to show allelism with the *pyd* and *wd* mutants. That it has not been detected so far in progeny tests of such plants may be due to the difficulty of detecting this mutant in the seedling stages, which have been used almost exclusively for detecting the newly produced mutants.

The known conditions connected with the origin and action of the spotted-leaf phenotype are instructive in a consideration of the problems associated with some previously studied types of interrelated but independently inherited mutant factors. A succession of a few relatively simple chromosomal aberrations may well account for some of these observed relations.

The Unexpected Appearance of a Number of Unstable Mutants

In the cultures that were grown to observe the mutations produced as a consequence of the breakage cycles of chromosome 9, an unusual and unexpected series of new mutants appeared, characterized by types of instability known in genetic literature as mutable genes, variegation, or mosaicism. Although maize has been ex-

tensively investigated, the appearance of "mutable genes" has been relatively rare. In the present comparatively restricted study of the progeny of plants having newly broken chromosomes 9, fourteen distinctly new expressions of instability of genic action have been isolated, although more types have been observed. Six of these have received at least preliminary investigation. It is not possible, however, to follow the genetic or cytological factors associated with all these new variegated types, because of the extensive amount of time and labor involved in the study of any one. The plant characters that may be most readily detected in variegated or mosaic patterns involve some form of color change in a tissue. Thus, most of the variegating types that have been recognized involve either anthocyanin or chlorophyll pigments. It should be emphasized that no one of these variegating mutants has received more than a preliminary study; however, a brief description of four of these mutable types will serve to illustrate the nature of the phenomena and the problems involved.

Variegated white seedling. This mutant first appeared in the progeny of a self-pollinated plant in which both chromosomes 9 had been involved in the chromosome type of breakage cycle in the very young embryonic stage. This first progeny consisted of 108 normal green seedlings and 19 white seedlings. In the white seedlings, sectors of pale-yellow or of normal-green tissue were present. Each pale-yellow or green sector was sharply delimited; all the plastids in the pale-yellow cells were yellow and all the plastids in the green cells were normal green. The size of the pale-yellow sectors varied from large, indicating an early mutation from white to pale-yellow, to very small, indicating a late mutational change to pale-yellow. Most of the green sectors were small, indicating that the mutations to green usually occur late in the life of the tissue. A few early mutations occurred, however, as there were a few rather wide green sectors in the leaves of some of these white seedlings. The rate of mutation to pale-yellow and to green in any one seedling was relatively constant; in general, each seedling was distinguished by one particular rate of mutational change. In some of the seedlings, distinct sectors were present in which the rate of mutation had increased considerably, although within these well defined sectors themselves uniform rates of mutation were discernible. This variegating mutant behaved as a simple recessive upon further testing. The phenotypic expression of the variegation—that is, white, pale-yellow, and green—is strikingly similar to the allelic series composed of *wd, pyd,* and normal-green associated with overlapping deficiencies at the tip of the short arm of chromosome 9. A further similarity to the *wd* mutant is apparent: when plants heterozygous for this variegating white mutant are selfed, a number of kernels with defective embryos are produced. If the proportion of kernels with defective embryos is high on any one ear, the expected proportion of variegated white seedlings is correspondingly lowered. In each case, the total number of defective embryos plus white-variegated seedlings approximates the one-fourth expected in the progeny. This behavior is quite similar to that of the *wd* mutants that have been extensively studied. Nevertheless, when this mutant is combined with the *wd* mutants, only normal-green seedlings and plants are produced. Furthermore, tests have shown that this mutant is not located in the short arm of chromosome 9.

Variegated light-green. The recessive light-green mutant appeared in the progeny of a self-pollinated plant that had received a chromosome 9 which had been under-

going the chromatid type of breakage cycle. Approximately one-fourth of the seedlings in this progeny were very light green. All the light-green seedlings showed a number of sectors of normal-green tissue, most of them appearing as fine green streaks in the light-green leaf. In the cells that are light green, the plastid size and number are normal but the chlorophyll color in all plastids is light green. In the sectors of normal tissue, the chlorophyll in all plastids is normal green in color. In any one seedling, the mutations to normal green occur at an approximately constant rate. Very wide differences, however, appear among the various seedlings; some have very high rates of mutation, others have very low rates, with many seedlings showing gradations between these extremes. These definite rates of mutational change from light green to normal green are maintained throughout the life of an individual plant, except for distinct sectors, presumably arising from individual cells, that show an increased rate or a reduced rate of mutation, respectively, over that of the plant as a whole. In a number of cases, two distinctly delimited but adjacent sectors showed inverse relations in their mutation rates. In one sector the rate of mutation, as expressed by the number of green streaks in the leaf tissue, was greatly increased, whereas in the sister sector the number of green streaks was greatly reduced. The positions of these twin sectors in the stalk and leaf suggested that they arose from two sister cells of the growing point. Such striking changes in the rate of mutation of cells derived from a single somatic cell of a plant have characterized all the variegating types that have been under study. The yet-unknown alteration that occurs in this cell determines the rates of mutation of the recessive mutant to a dominant allele in all cells that arise from it, until another such change suddenly occurs. The frequent appearance of twin sectors showing inverse rates of mutation leads to the suggestion that the controlling factor for future mutational occurrences may be altered or segregated at a somatic mitosis so that the mutations that will occur in the cells arising from one daughter cell are increased over the potential rate of the mother cell; conversely, the mutations that will occur in the cells arising from the other daughter cell are reduced.

The light-green character is inherited as a simple recessive. In individual cultures, the F_2 ratios frequently approximate three normal-green plants to one light-green plant. This usually occurs when the mutation rates in the light-green plants are uniformly low. However, in some F_2 cultures segregating light-green plants with low mutation rates, the ratios were distinctly aberrant. When the mutation rates are high in the light-green plants of an F_2 culture, a deficiency in the light-green class frequently occurs. This suggests that mutations of the light-green locus to normal green have occurred at a relatively high rate in the F_1 heterozygous plant and result in an increase in the relative number of gametes carrying normal green. It is possible that the light-green locus may occasionally mutate to intermediate alleles, as several distinctive types of the light-green mutant have appeared in a few of the cultures.

The luteus mutant. The recessive luteus mutant is one of the more interesting and complex of the variegating mutants that have appeared in this study. The luteus character is associated with the presence of small yellowish plastids. The luteus seedlings are, therefore, bright yellow. From one original plant that had received a chromosome 9 which had undergone the chromatid type of breakage cycle, two kernels were removed and plants were grown from them. Each plant was self-

pollinated. In the progeny of one plant, yellow (luteus) seedlings segregated. These showed no variegation. In the progeny of the second plant, yellow seedlings segregated but many were highly variegated for normal-green tissue. The nonvariegated luteus seedlings from the first plant were inviable, but the more highly variegated luteus seedlings from the second plant were viable, presumably because of the extensive amounts of normal-green tissue. Further tests showed that the nonmutating or stable luteus and the highly mutating luteus represent two extreme states of one particular locus. The stable luteus is only relatively stable and, in the tests so far conducted, has only rarely become mutable again.

In F_2 progeny tests, stable luteus usually segregated quite normally. From 52 self-pollinated F_1 plants there were obtained 2306 normal-green seedlings, 773 nonvariegated luteus seedlings, and only 3 variegated luteus seedlings. These 3 variegated luteus seedlings appeared in three different cultures. In one single F_2 culture, 231 normal-green seedlings, 51 nonvariegated luteus seedlings, and 10 variegated luteus seedlings appeared. In this case, it is probable that in a cell of the F_1 heterozygous plant the stable luteus suddenly became unstable and formed a sector of mutating luteus. In contrast with the stable luteus, the highly variegating luteus is distinguished by great instability of the luteus locus. It may mutate to normal green but also, phenotypically at least, to several distinctive intermediate alleles.

Plants heterozygous for the highly stable luteus and a mutating luteus may exhibit various rates of mutability in a manner quite similar to that observed among the variegated white seedlings and the variegated light-green plants. A distinctive and relatively constant rate of mutation from luteus to green is apparent in each individual seedling. These rates range from very low, with only a few small green streaks on a leaf, to very high, where the leaf appears to be almost mottled because of the many mutations that have occurred. Most of the mutations occur late in the development of the tissues, but a few occur earlier and give rise to wide sectors of light-green or normal-green tissue. In a manner quite similar to that found in mutable white and light-green, distinctive sectors appear in these plants with decidedly altered rates of mutation, either increased or decreased. Adjacent sectors (twin sectors) also occur, one showing an increased rate of mutation, the other a reduced rate of mutation as compared with that in the plant as a whole.

A further complexity also appeared. It was first clearly recognized in the progeny resulting from a cross of plants that were heterozygous for a stable luteus locus and its wild-type allele by the variegated luteus plants that had both a mutating and a stable luteus locus. Because the F_1 plants resulting from the combination of either a stable luteus or a mutating luteus with a normal wild-type allele have been normal green in appearance, one could expect to find normal-green plants, variegated luteus plants, and nonvariegated luteus plants in the progeny of the above cross. These three types of plant appeared, but, in addition, a distinctly new type of variegated plant was present. These plants were green but showed fine streaks of luteus. The numbers of such streaks varied greatly among the different plants. When very many streaks were present, they occurred in well defined sectors within the leaf. In a few of these plants, wide sectors of luteus were present. Within some of these luteus sectors, mutations to normal green occurred, each sector showing its own particular rate of mutation from luteus to green. Because these plants were mainly

green, with fine streaks of luteus, they were designated "streaked." In their patterns, the streaked plants resembled the variegated luteus plants, but they were reverse images—that is, negatives (luteus streaks in green tissue)—of a positive (green streaks in luteus tissue) variegation pattern. This suggests that some of the mutations of luteus to green are unstable and revert to luteus by processes similar to those that give rise to the dominantly directed mutations. As a working hypothesis, to be considered as tentative only, it may be conceived that the locus concerned may be present in a very stable recessive state, rarely mutating to dominant, or in a very stable dominant state, rarely mutating to luteus; or that it may be present in recessive or dominant states with various intermediate rates of mutability. Furthermore, changes in the stability of any one state may occur suddenly, following some yet-unknown event that probably takes place during a mitosis.

The chromosome-breakage variegation. The most unexpected expression of variegation that has appeared in these investigations is associated with the occurrence, in many somatic cells, of breakage in chromosome 9 that takes place at a particular locus in the short arm of this chromosome. This breakage results in the formation of an acentric fragment and the subsequent elimination of this fragment at a somatic anaphase. This phenomenon first appeared in the progeny of a self-pollinated plant that had started development with its two chromosomes 9 undergoing the chromosome type of breakage cycle, although healing of the broken ends had occurred in early embryogeny. A few of the kernels on the ear of this plant exhibited a type of aleurone color variegation that had not previously been observed. In this plant, one newly broken chromosome 9 carried the factors *I* (dominant inhibitor of aleurone color) and *Wx* (starch in endosperm stains blue with iodine). Its homologue carried the recessive alleles *i* (colored aleurone) and *wx* (starch stains red with iodine). The aberrant kernels were recognized in the heterozygous class that had received both the *i*- and the *I*-carrying chromosomes. According to their genetic constitution, such kernels should be colorless. The aberrant kernels were conspicuous because of the presence of colored (*i*) areas. In some kernels, there were well defined sectors that exhibited a uniform pattern of small *i* spots. The pattern in any one such sector was distinguished by the number of *i* spots, their uniform distribution within the sector, and their relatively similar size. The patterns in these sectors suggested that the *I* factor, carried by one chromosome 9, had been systematically eliminated from some cells and that, in each sector, this had occurred at a particular rate and at a particular stage in the development of the endosperm tissue. Subsequent testing indicated that the *I*-carrying chromosome also possessed the minute terminal deficiency that is responsible, when homozygous, for the appearance of white seedlings (the *wd* mutant). Consequently, green and white seedlings segregated in the progeny of this self-pollinated plant, the white seedlings arising mainly from the *I* kernels.

To investigate the nature of the phenomenon associated with the appearance of the *i* spots, the green plants arising from some of the *I* kernels were grown in the summer of 1945. In many of these plants, fine streaks of white tissue, rather uniformly distributed over the leaf, were observed. In some plants, there were only a few such non-chlorophyll-bearing white streaks; in others, there were more; in one plant, the number of white streaks was relatively large. In most of the plants, dis-

tinct sectors were present in which the number of uniformly distributed white streaks was greatly increased or decreased over that of the plant as a whole. In these aspects, the patterns of variegation resembled those observed in the three previously described variegating types.

In agreement with the presence of the *i* spots in some of the *I* kernels, the presence of white streaks in the leaves of many of the plants again suggested that a segment of chromatin of one chromosome 9 was being systematically eliminated in somatic cells. Elimination from one chromosome 9 of the chromatin segment that covers the white-producing deficiency in the other chromosome 9 would allow the non-chlorophyll-producing effect of this deficiency to be expressed. According to this interpretation, it would be necessary for the streaked plants to be heterozygous for the *wd* mutant. Tests confirmed the presence of the *wd* mutant in all the streaked plants.

The white-streaked plants used for further crosses carried *wd, I, Bz, Wx* in one chromosome 9 and *Wd* (i.e., chromatin segment covering the white-producing deficiency), *i, Bz, wx* in the homologous chromosome 9. Pollen of these streaked plants was placed on silks of plants carrying various recessive mutants located in the short arm of chromosome 9. One such cross, to *i bz wx,* was particularly instructive. The locus of *bz* (bronze, very light aleurone color) lies between those of *I* and *Wx*. The *Wx* locus is closest to the centromere, but the loci of *Wd, I, Bz,* and *Wx* are all included in the distal two-thirds of the short arm. On examination of the types of kernel resulting from this cross, it was again apparent that some kind of chromatin loss, involving a segment of the short arm of chromosome 9 that included at least the *I, Bz,* and *Wx* loci, was responsible for the variegation patterns observed in some of the resulting kernels. In these crosses, the behavior of chromosome 9 in endosperm development could be critically evaluated, from a genetic point of view, only in those kernels that had received an *I*-carrying chromosome from the white-streaked parent. The aleurone variegation pattern, involving the alleles *I* and *i, Bz* and *bz,* or *Wx* and *wx* in the kernel, and the white-streaked pattern, involving *Wd* and *wd* in the plant, clearly differed from the previously known chromatin-loss patterns associated with ring chromosomes or with the chromosome or chromatid bridge cycles. In all appropriate crosses, an additional fact was noted. Somatic elimination of chromatin was observed mainly in those *I* kernels that had received an *I wx* chromosome—that is, a chromosome 9 resulting from a crossover between the loci of *I* and *Wx* in the white-streaked parent plant. Relatively few *I Wx* chromosomes were undergoing chromatin elimination. This would suggest that if a locus in chromosome 9 is responsible for the somatic elimination of segments of chromatin, its position in the chromosome is proximal but close to the *wx* locus. This would place the chromatin-elimination locus in the *Wd i wx* chromosome of the streaked parent plant. To illustrate the present knowledge of the nature of this chromatin-elimination phenomenon, the subsequent behavior of one *I wx* chromosome will be briefly described.

In the cross of a white-streaked plant with the constitution *wd I Wx/Wd i wx* to a normal plant homozygous for *Wd i wx,* an *I wx* kernel, highly speckled with *i,* was removed and germinated. A normal-appearing, nonstreaked plant arose from this kernel. The plant was self-pollinated. On the resulting ear, there again appeared *I* kernels that were highly spotted with *i.* This time, however, in contrast with the above crosses, a large number of these

kernels were present. The seedlings grown from the kernels of this ear were classified into three distinct types: green seedlings with few or no white streaks, green seedlings highly streaked with white, and totally white seedlings. The proportions of these seedling types arising from the nonspotted or only very moderately spotted *I* kernels, the heavily spotted *I* kernels, and the *i* kernels are given in the accompanying table.

TYPES OF SEEDLINGS ARISING FROM THREE CLASSES OF KERNELS FOLLOWING SELF-POLLINATION OF A WD I/WD I PLANT

CLASS OF KERNELS	NO. OF SEEDLINGS		
	Green	White-streaked	White
I	63	5	33
Highly spotted	21	4	1
i	70	1	0

All the 10 highly streaked seedlings, and one obviously peculiar nonstreaked seedling arising from an *i* kernel, were transplanted to the field in the summer of 1946. The white streaks continued to appear in the leaves as the heavily streaked seedlings developed into mature plants. The patterns were similar to those of the original white-streaked plants, but the total number of such fine white streaks was enormously greater. In contrast, the nonstreaked seedling remained nonstreaked throughout its development.

To observe whether chromatin losses could be seen in somatic mitoses of tissues that were relatively late in their developmental period, whole mounts of the young membranous glumes in the florets of the tassels of the streaked plants and the nonstreaked plant were stained and examined. In all examined glumes, many cells showed, besides their main nucleus, a very small micronucleus or a very small deep-staining pycnotic chromatin body. The majority of the anaphase figures appeared normal, but in some late anaphases one fragment or two identical-sized fragments were observed. Time did not allow a detailed study of the sequence of events or of the frequencies or types of fragment formation. An examination of the sporocytes of the anthers of all 11 plants, however, has thrown some light on the nature of the fragmentation phenomenon.

The chromosome-9 constitution at pachytene in the microsporocytes of all 11 plants was examined. In all 10 white-streaked plants, many of the sporocytes showed the presence of a morphologically normal chromosome 9 and a chromosome 9 carrying the minute *wd*-producing deficiency. In the nonstreaked plant, two morphologically normal chromosomes 9 were present in many of the sporocytes; the *wd*-producing deficiency was not present in this plant. In the individual anthers of all 11 plants, however, the sporocytes exhibited mosaicism for a deficiency of a long segment of the short arm of chromosome 9. In many sporocytes, loss of the segment from one chromosome 9 had occurred in a recent premeiotic mitosis, although in some cases losses had occurred earlier to give rise to a relatively large cluster of related sporocytes each having a long deficiency in one chromosome 9. In a number of sporocytes, a small deep-staining pycnotic chromatin body was present in the cytoplasm. In many of these cells, in turn, the constitution of the chromosome-9 bivalent could be analyzed. In the clearest cases, it could be determined with certainty that one chromosome 9 was deficient for a large segment of the short arm. Thus, a correlation could be obtained between the presence of the pycnotic chromatin body in the cytoplasm of a cell and the absence of a long segment from the short arm of

one chromosome 9 of the same cell. The cytological studies must be greatly extended in order to clarify and complete the needed information, but it is quite clear that the positions of "breakage" in chromosome 9 do not occur at random. In a large number of figures, it could be determined that the segment deleted from the nucleus at an anaphase included the terminal two-thirds of the short arm; in other words, the break, in each case, occurred at a position approximately one-third the distance from the centromere. The acentric segment that resulted from such a break was subsequently lost to the nuclei during a mitosis. Not all were immediately lost, however, for in some sporocytes a bivalent acentric terminal segment of chromosome 9 was present along with the normal and deficient chromosomes 9. Various types of synaptic association were observed between the acentric segments and the short arm of the unbroken chromosome 9. In a number of sporocytes, what appeared, on rapid examination, to be a normally synapsed bivalent chromosome 9 proved to be otherwise. In the short arm of one of the chromosomes of the bivalent, a break was unquestionably present at a position approximately one-third the distance from the centromere. Very occasionally, cells were observed with other abnormalities of chromosome 9. It is possible that these resulted from secondary effects of the primary breakage process, but any conclusions must await a more extensive study. The nature of the process that is responsible for this most unusual type of chromosome "breakage" is not understood. Two lines of evidence indicate that the locus of breakage is not a "weak" spot in the chromosome that is subject to breakage following unusual tension on the chromosome. If a chromosome with this locus is subjected to the chromatin bridge cycles, breaks occur at various positions in the short arm regardless of this locus. Furthermore, the presence of distinct sectors, each one with its own uniform rate of breakage of the chromosome at this locus, cannot readily be interpreted on the basis of a structurally weak spot in the chromosome. It may be suspected that the "breakage" occurs during the chromosome reduplication process as a consequence of some yet-undetermined abnormality that is present at this one particular locus in the chromosome. Such a modified locus should be subject to genetic analyses. Preliminary genetic evidence derived from plants that were heterozygous for various mutants in the short arm of chromosome 9, as well as heterozygous for this altered locus, have placed this locus in a position which conforms to the position that had been determined cytologically.

It should be mentioned that the single non-white-streaked plant was unusual because of the very high rate of breakage that occurred in it. Both chromosomes 9 underwent the breakage phenomenon, but only occasionally did breakage involve both chromosomes 9 in a single nucleus. When this occurred in a meiotic nucleus, it produced unquestionable evidence for the precise localization of the breaks in chromosome 9. A genotypically complete chromosome-9 bivalent was present at pachytene, but in the form of two entirely detached segments. The homologously associated distal two-thirds of the short arms of both chromosomes 9 formed one segment; the other segment was composed of the homologously associated long arms, the centromeres, and the proximal third of the short arms. It could readily be observed that the break had occurred at the very same locus in each chromosome 9. This was the same locus of breakage that had been observed when only one of the two chromosomes 9 had been broken. It is

possible that in this plant the excessive rate of loss from somatic nuclei of the large segment of chromosome 9 was responsible for its selection as an aberrant seedling. The leaf tissue of the seedling probably was heavily mosaic for different genomic complements of chromosome 9. This may have been responsible for its atypical appearance.

The cytological observations of breakage of chromosomes 9, predominantly at a particular locus, and of the subsequent elimination of the acentric segment that results, are consistent with the genetic observations. This segment carries the loci of *Wd*, *I*, *Bz*, and *Wx*, in this order. If, in heterozygous plants, these dominant alleles are carried by the chromosome that is undergoing breakage in various somatic cells, simultaneous losses of these dominant alleles should occur following deletion of the acentric fragment from a nucleus. In the kernels, such simultaneous losses of the *I*, *Bz*, and *Wx* loci have been observed. It is apparent that the loss of the *Wd* locus accounts for the white (*wd*) streaks in the heterozygous plants.

Although the factors responsible for this breakage phenomenon are not understood, nevertheless the factors that control the frequency and the timing of such occurrences are similar to those that control the frequency and timing of dominantly directed mutations in the variegation types previously described. In the case of the chromosome-breakage variegation just described, however, the breakage itself corresponds to the "gene" mutations observed in the other variegations. Possibly the resemblance is more than coincidental, in that the underlying phenomena are basically similar.